

We Claim:

1. A modular molecular clasp comprising a plurality of heterologous components including:

5 a molecular recognition element;

an effector; and

a transducer, constructed such that said transducer facilitates allosteric alteration of said modular molecular clasp in response to ligand binding to said molecular recognition element, producing a detectable change in an activity of said effector.

10 2. A modular molecular clasp comprising:

two single chain antibody domains together forming a ligand binding site;

an effector; and

15 a transducer linking conserved regions of the single chain antibody domains, wherein said modular molecular clasp is constructed such that allosteric alteration of said modular molecular clasp is facilitated in response to ligand binding to said molecular recognition element, producing a detectable change in an activity of said effector.

20 3. A modular molecular clasp comprising a plurality of heterologous components including:

a molecular recognition element;

an effector; and

25 a transducer comprising a pair of polypeptides that form a noncovalently bound complex in response to ligand binding to said molecular recognition element, constructed such that the transducer facilitates allosteric alteration of said modular molecular clasp, producing a detectable change in an activity of said effector.

4. A modular molecular clasp comprising a plurality of heterologous components including:

a molecular recognition element;

an effector; and

a transducer comprising a pair of polypeptides that form a noncovalently bound complex in the absence of ligand binding to said molecular recognition element,  
5 constructed such that the transducer facilitates allosteric alteration of said modular molecular clasp, producing a detectable change in an activity of said effector.

5. A modular molecular clasp comprising a plurality of heterologous components including:

10 a molecular recognition element, wherein said molecular recognition element comprises two protein domains together forming a ligand binding site, is derived from a protein superfamily and comprises a portion which is conserved among members of said protein superfamily;

an effector; and

15 a transducer which links said conserved portions within said molecular recognition element, constructed such that said transducer facilitates allosteric alteration of said modular molecular clasp in response to ligand binding to said molecular recognition element, producing a detectable change in an activity of said effector.

6. A modular molecular clasp comprising a plurality of heterologous components including:

20 a molecular recognition element, wherein said molecular recognition element is derived from a protein superfamily and comprises a portion which is conserved among members of said protein superfamily;

an effector; and

25 a transducer, constructed such that said transducer binds to said conserved portion in the absence of ligand binding to said molecular recognition element but is displaced upon ligand binding to said molecular recognition element, producing a detectable change in an activity of said effector.

7. The modular molecular clasp of claim 1, wherein the energy produced from ligand binding to said molecular recognition element is insufficient in itself to induce allosteric alteration of said molecular recognition element.

5 8. The modular molecular clasp of claim 1, wherein said transducer comprises a pair of polypeptides that form a noncovalently bound complex in response to ligand binding to said molecular recognition element.

9. The modular molecular clasp of claim 1, wherein said transducer comprises a pair of polypeptides that form a noncovalently bound complex in the absence of ligand binding to said molecular recognition element.

10 10. The modular molecular clasp of any one of claims 8 or 9, wherein said transducer comprises a pair of anti-parallel coils.

11. The modular molecular clasp of any one of claims 8 or 9, wherein said transducer comprises a pair of strands from a beta-hairpin structure.

15 12. The modular molecular clasp of any one of claims 8 or 9, wherein said transducer comprises an SH3 domain-peptide pair.

13. The modular molecular clasp of any one of claims 8 or 9, wherein said molecular recognition element comprises less than 50 amino acid residues.

14. The modular molecular clasp of any one of claims 8 or 9, wherein said molecular recognition element comprises less than 25 amino acid residues.

20 15. The modular molecular clasp of claim 1, wherein said molecular recognition element comprises two protein domains together forming a ligand binding site.

25 16. The modular molecular clasp of claim 15, wherein said molecular recognition element is selected from the group consisting of single chain antibodies (scFv), single chain T cell receptors and single chain MHC molecules.

17. The modular molecular clasp of claim 15, wherein said molecular recognition element comprises a single chain antibody.

18. The modular molecular clasp of claim 15, wherein said molecular recognition element is derived from a protein superfamily and comprises a portion which is conserved among members of said protein superfamily.

19. The modular molecular clasp of claim 18, wherein said transducer links  
5 said conserved portions within said molecular recognition element.

20. The modular molecular clasp of claim 15, wherein said transducer comprises less than 20 amino acid residues.

21. The modular molecular clasp of claim 1, wherein said molecular recognition element is derived from a protein superfamily and comprises a portion  
10 which is conserved among members of said protein superfamily; and wherein said transducer binds to said conserved portion in the absence of ligand binding to said molecular recognition element but is displaced upon ligand binding to said molecular recognition element.

22. The modular molecular clasp of claim 21, wherein said transducer moiety  
15 comprises less than 20 amino acid residues.

23. The modular molecular clasp of any one of claims 1, 2, 3, 4, 5, or 6, wherein said transducer is operative with a plurality of distinct molecular recognition elements.

24. The modular molecular clasp of any one of claims 1, 2, 3, 4, 5, or 6,  
20 wherein said effector is operative with a plurality of distinct transducers and a plurality of distinct molecular recognition elements.

25. The modular molecular clasp of any one of claims 1, 2, 3, 4, 5, or 6, wherein said effector is selected from the group consisting of fluorophores, complementary enzyme fragments, inorganic nanoparticles, transcriptional activators,  
25 transcriptional repressors, radioactive molecules, radioactive molecular aggregates and enzyme-peptide inhibitor complexes.

26. The modular molecular clasp of claim 25, wherein said fluorophore is selected from the group consisting of green fluorescent protein or fluorescent variants thereof and DS Red.  
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27. The modular molecular clasp of claim 25, wherein said fluorophore is a fluorescent label selected from the group consisting of: Alexa Fluor 350, Alexa Fluor

488, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660 and Alexa Fluor 680, AMCA, AMCA-S, BODIPY FL, BODIPY R6G, BODIPY TMR, BODIPY TR, BODIPY 530/550, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY 630/650, BODIPY 650/665,  
 5 Carboxyrhodamine 6G, carboxy-X-rhodamine (ROX), Cascade Blue, Cascade Yellow, Cy3, Cy5, Cy3.5, Cy5.5, Dansyl, Dapoxyl, Dialkylaminocoumarin, 4',5'-Dichloro-2',7'-dimethoxy-fluorescein, DM-NERF, Eosin, Erythrosin, Fluorescein, FAM, Hydroxycoumarin, IRD40, IRD 700, IRD 800, JOE, Lissamine rhodamine B, Marina Blue, Methoxycoumarin, Naphthofluorescein, Oregon Green 488, Oregon Green 500,  
 10 Oregon Green 514, Pacific Blue, PyMPO, Pyrene, Rhodamine 6G, Rhodamine Green, Rhodamine Red, Rhodol Green, 2',4',5',7'-Tetra-bromosulfone-fluorescein, Tetramethyl-rhodamine (TMR), Carboxytetramethylrhodamine (TAMRA), Texas Red, Texas Red-X, squaraine dye Sq635, and squaraine dye Sq660.

15           28.     The modular molecular clasp of claim 25, wherein said effector comprises a fluorophore that supports Fluorescence Resonance Energy Transfer.

              29.     The modular molecular clasp of claim 25, wherein said effector comprises a fluorophore that supports fluorescence quenching.

20           30.     The modular molecular clasp of claim 25, wherein said effector comprises a fluorophore and a bioluminescent protein, the combined use of which supports Bioluminescence Resonance Energy Transfer.

              31.     The modular molecular clasp of claim 25, wherein said effector comprises complementary enzyme fragments that exhibit reduced catalytic activity  
 25 when spaced apart and increased catalytic activity when disposed together.

              32.     The modular molecular clasp of claim 1, wherein said molecular recognition element is derived from a naturally occurring polypeptide.

              33.     The modular molecular clasp of claim 1, wherein said molecular recognition element is an artificial polypeptide.

30           34.     The modular molecular clasp of claim 1, wherein said molecular recognition element is selected from the group of molecular recognition element superfamilies consisting of single chain antibodies (scFv), single domain antibodies (VHH), lipocalins, single chain T cell receptors and single chain MHC molecules.

35. The modular molecular clasp of claim 1, wherein said molecular recognition element is selected from the group consisting of molecular recognition element superfamilies consisting of anticalins<sup>TM</sup>, affibodies<sup>TM</sup>, and trinetin<sup>TM</sup>.

36. The modular molecular clasp of claim 1, wherein said molecular recognition element comprises a VH chain specific for a ligand of interest, or a portion thereof.

37. The modular molecular clasp of claim 1, wherein said molecular recognition element comprises a VL chain specific for a ligand of interest, or a portion thereof.

38. The modular molecular clasp of claim 1, wherein molecular recognition element comprises about 1-220 amino acid residues.

39. The modular molecular clasp of claim 1, wherein molecular recognition element comprises about 1-150 amino acid residues.

40. The modular molecular clasp of any one of claims 1, 2, 3, 4, 5, or 6, further comprising a fusion partner domain.

41. The modular molecular clasp of claim 40, wherein said fusion partner domain is a targeting sequence which localizes said modular molecular clasp to an intracellular compartment.

42. The modular molecular clasp of claim 40, wherein said fusion partner domain is a targeting sequence which localizes said modular molecular clasp to a cellular membrane.

43. The modular molecular clasp of claim 40, wherein said fusion partner domain is suitable for immobilizing said modular molecular clasp on a solid surface.

44. The modular molecular clasp of claim 40, wherein said fusion partner domain facilitates purification or isolation of said modular molecular clasp.

45. The modular molecular clasp of claim 40, wherein said fusion partner domain is capable of modifying the solubility of the modular molecular clasp.

46. An isolated nucleic acid molecule encoding the modular molecular clasp of any one of claims 1, 2, 3, 4, 5, or 6.

47. A method of producing a modular molecular clasp comprising culturing a host cell comprising the isolated nucleic acid molecule of claim 46 under conditions in which the nucleic acid molecule is expressed; and isolating the modular molecular clasp from the host cell or a host cell culture medium.

5 48. An isolated host cell comprising the isolated nucleic acid molecule of claim 46.

49. A transgenic animal comprising the isolated nucleic acid molecule of claim 46.

10 50. A transgenic plant comprising the isolated nucleic acid molecule of claim 46.

51. A method of designing a modular molecular clasp comprising:

selecting a transducer comprising a pair of polypeptides, such that said polypeptides have sufficient affinity for each other to form a noncovalently bound complex in response to ligand binding to a molecular recognition element; and

15 positioning said transducer forming a modular molecular clasp,

wherein said transducer is positioned such that it facilitates allosteric alteration of a modular molecular clasp in response to ligand binding to said molecular recognition element, producing a detectable change in an activity of an effector.

52. A method for detecting the presence or absence of a ligand comprising:

20 contacting a solution suspected of containing a ligand with the modular molecular clasp of any one of claims 1, 2, 3, 4, 5, or 6 under conditions suitable for binding of said ligand to said molecular recognition element, and

detecting a change in an activity of said effector, thereby detecting the presence or absence of a ligand.

25 53. The method of claim 52, wherein the presence or absence of the ligand is an indicator of a disease state.

54. The method of claim 53, wherein the ligand is a marker of an infectious agent, a prion, a parasite, or a transformed cell.

55. The method of claim 53, wherein the ligand is a marker of a virus, a bacterium or a fungus.

56. A method of identifying a modulator of a ligand of interest, comprising:  
providing the molecular clasp of any one of claims 1, 2, 3, 4, 5, or 6;  
5 contacting said molecular clasp with a test compound and a ligand of interest;  
and,

detecting a change in an activity of said effector, thereby determining whether said test compound can modulate ligand binding to said molecular clasp.

57. A method of detecting the presence of a contaminant in a sample  
10 comprising:

providing the modular molecular clasp of any one of claims 1, 2, 3, 4, 5, or 6;  
wherein said molecular recognition element is capable of binding with said contaminant;

contacting said modular molecular clasp with a sample suspected of containing said contaminant; and,

15 detecting a change in an activity of said effector, thereby detecting the presence of a contaminant in a sample.

58. An array of modular molecular clasps, the array comprising:

a solid support having at least a first surface; and  
20 a plurality of modular molecular clasps, as defined in any one of claims 1, 2, 3, 4, 5, or 6, attached to the first surface of said solid support, wherein each of said modular molecular clasps is attached to the surface of said solid support in a different pre-defined region.

59. The array of claim 58, wherein the array comprises at least 1,000  
25 different modular molecular clasps attached to the first surface of said solid support.

60. The array of claim 58, wherein the array comprises at least 10,000 different modular molecular clasps attached to the first surface of said solid support.



61. The array of claim 58, wherein the modular molecular clasps are attached to the first surface of said solid support at a density of 100 modular molecular clasps/cm<sup>2</sup>.

5 62. The array of claim 58, wherein each of the different pre-defined regions is physically separated from each of the other different regions.

63. The array of claim 58, wherein said solid support is planar.

64. The array of claim 58, wherein said solid support is non-porous.

65. The array of claim 64, wherein said non-porous solid support is glass.

10 66. The array of claim 58, wherein said modular molecular clasps are immobilized to said solid support via a linker.

67. The array of claim 58, wherein said plurality of modular molecular clasps comprise different molecular recognition elements.

15 68. A method for treating a subject suffering from a disease comprising administering to the subject a therapeutically effective amount of a Modular Molecular Clasp comprising a molecular recognition element capable of binding to a disease marker on the surface of a cell and an effector comprising a non-therapeutic prodrug and an enzyme capable of converting the non-therapeutic prodrug to a therapeutic drug, thereby treating a subject suffering from a disease.

20 69. A method for treating a subject suffering from a disease comprising:  
administering to the subject a therapeutically effective amount of a Modular Molecular Clasp comprising a molecular recognition element capable of binding to a disease marker on the surface of a cell and an effector comprising an enzyme capable of converting a non-therapeutic prodrug to a therapeutic drug, and  
25 administering to a subject a therapeutically effective amount of a non-therapeutic prodrug, thereby treating a subject suffering from a disease.